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PINDOLOL DECREASES PLASMA ANGIOTENSIN CONVERTING ENZYME ACTIVITY IN YOUNG SPONTANEOUSLY HYPERTENSIVE RATS

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INTRODUCTION.

The renin-angiotensin system is controlled by a number of factors, among which beta-adrenoceptors are well understood. In turn, the antihypertensive action of beta-adrenoceptor blockers may be partially due to antagonism of the renin-angiotensin system (1). Alterations of the renin-angiotensin system occur in spontaneously hypertensive rats (SHR). These animals show low angiotensin converting enzyme (ACE) activity in plasma (2). It has been postulated that plasma ACE is originated in lung, although endothelial cells in peripheral vessels could also make a contribution (3). Studies have shown that angiotensin II locally produced in arterial tissues by ACE might be important in maintaining vascular tone (4).

We studied the effects of pindolol, a non-selective beta-adrenoceptor blocker and widely used antihypertensive drug, on plasma, lung and mesenteric artery ACE of young (4 to 5-week-old, pre or early hypertensive) and adult (24 to 25-week-old) SHR.

METHODS.

Pindolol was given orally for 7 days once a day at a dose of 10 mg/kg body weight to male, 4- and 24-week-old SHR and Wistar Kyoto (WKY) rats. Animals were decapitated 24 hr after the last treatment. ACE activity was measured by a radiochemical method (5). Lung and mesenteric arteries were homogenized in 0.1 M potassium phosphate buffer pH 8.0, by glass to glass tissue grinders. The homogenate was centrifuged at 130,000 x g for 30 minutes, yielding a pellet and a supernatant. The supernatant was considered to be a loosely-bound (soluble) ACE containing fraction (F_1). The pellet was resuspended in the buffer, containing 0.5% Triton X-100, ultrasonicated, and frozen-thawed 3 times in order to solubilize the particulate enzyme (6). The homogenate was centrifuged at 10,000 x g for 30 minutes; the supernatant was referred as the F_2 fraction (particulate).

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Table 1. EFFECT OF PINDOLOL ON PLASMA ACE ACTIVITY

	SHR-CONTROL	SHR-PINDOLOL	WKY-CONTROL	WKY-PINDOLOL
Young	2.40 ± 0.16**	1.61 ± 0.12 *	3.42 ± 0.28	3.32 ± 0.34
Adult	0.66 ± 0.10**	0.72 ± 0.08	1.06 ± 0.14	1.12 ± 0.12

n:5, * $p<0.01$ compared to SHR-control, ** $p<0.05$ compared to WKY-control, nmoles/μl plasma/hr

RESULTS.

Seven daily doses of pindolol decreased the heart weight in young SHR (from 500 ± 7 mg/100 g body weight in non-treated SHR to 436 ± 16 mg/100 g body weight in pindolol treated SHR (n:5,$p<0.05$). Plasma ACE was lower both in young and in adult SHR when compared to age-matched controls (Table 1). Seven daily doses of pindolol further decreased plasma ACE activity, an effect observed only in young, prehypertensive SHR (Table 1).

Kinetic studies showed that the decrease in plasma ACE in SHR after pindolol was due to the decrease of number of ACE molecules rather than to the change of the affinity for the substrate: Km (mM), SHR-pindolol:1.34, SHR-control:1.30, WKY-pindolol:1.40, WKY-control:1.42; Vmax (nmoles/μl/hr), SHR-pindolol:2.04, SHR-control: 3.18, WKY-pindolol:4.23, WKY-control:4.38.

In vitro studies showed that pindolol had no direct effect on plasma ACE activity, determined over pindolol concentrations ranging from $2x10^{-7}$ to $2x10^{-3}$M (results not shown).

ACE activity in F_2 fraction (particulate) of lung and mesenteric arteries in young SHR was significantly lower than that in WKY. No differences were observed after pindolol treatment in either SHR or WKY. In the F_1 fraction from lung, however, the ACE activity in SHR-pindolol treated was slightly decreased when compared to SHR-control and significantly decreased when compared to WKY-control (Table 2). A similar trend was found in F_1 from mesenteric arteries, although the differences were not statistically significant (Table 2).

DISCUSSION:

Plasma ACE activity was low in both young and adult SHR when compared with normotensive age-matched controls. Similarly, the F_2 (particulate) ACE activity was low in lung and mesenteric arteries of young SHR when compared with age-matched normotensive controls. These differences may be genetically determined and could be related to local alteration in angiotensin metabolism.

Only young, pre or early hypertensive SHR are sensitive to the effect of pindolol on plasma ACE. Beta-adrenoceptor blockers can clearly reduce blood pressure in SHR only when treatment is star-

TABLE 2. EFFECT OF PINDOLOL ON LUNG AND MESENTERIC ARTERY ACE ACTIVITY

	SHR-CONTROL	SHR-PINDOLOL	WKY-CONTROL	WKY-PINDOLOL
LUNG				
F_1	40 ± 5	26 ± 6 *	59 ± 9	61 ± 6
F_2	424 ± 22 *	409 ± 22 *	635 ± 64	602 ± 83
MESENTERIC ARTERY				
F_1	4.6 ± 0.5	3.4 ± 0.6	5.1 ± 0.5	5.2 ± 0.6
F_2	26.3 ± 2.0 *	34.2 ± 5.8	42.0 ± 4.1	40.8 ± 5.6

n:5, * $p<0.05$ compared to WKY-control, pmoles/μg protein/hr

ted in the young rats (7). Thus, the effect of pindolol on plasma ACE of SHR could be related to its therapeutic effects.

Pindolol treatment might also affect the sites of origin of plasma ACE. F_1 (soluble) ACE is slightly reduced by pindolol in young SHR, suggesting a role of beta-adrenoceptors in the regulation of the ratio of particulate to soluble ACE. Pindolol could produce these effects directly, or indirectly through its inhibitory effects on thyroid function. Particulate ACE is converted to a soluble form which is excreted from the endothelial cells into the blood (8), a process regulated by a chymotrypsin-like esteroprotease, which in turn is induced by triiodothyronine (9). It is of interest that pindolol effects occur only in young hypertensive animals. The susceptibility of early hypertensive rats to the effects of beta-adrenoceptor blockers on ACE activity might be correlated with the increased peripheral sympathetic activity observed in young SHR (10) or with alterations in membrane phospholipid metabolism in this model (11).

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